1

SEQUENCE LISTING

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 TANAKA, EISHI
 OIKAWA, TOSHIHIRO
- <120> NOVEL NITRILE HYDRATASE
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- <140> 10/539,560
- <141> 2005-06-16
- <150> PCT/JP03/016014
- <151> 2003-12-15
- <150> JP 2003-379280
- <151> 2003-11-10
- <150> JP 2002-368360
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- Asp Pro Glu Phe Lys Lys Arg Leu Leu Ala Asp Gly Thr Glu Ala Cys 65 70 75 80
- Lys Glu Leu Gly Ile Gly Gly Leu Gln Gly Glu Asp Met Met Trp Val\$85\$ 90 95
- Glu Asn Thr Asp Glu Val His His Val Val Val Cys Thr Leu Cys Ser 100 105 110

Cys Tyr Pro Trp Pro Val Leu Gly Leu Pro Pro Asn Trp Phe Lys Glu 115 120 125

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Ala Phe Ala Met Phe Pro Ala Thr Phe Arg Ala Gly Phe Met Gly Leu 35 40 45

Asp Glu Phe Arg Phe Gly Ile Glu Gln Met Asn Pro Ala Glu Tyr Leu 50 55 60

Glu Ser Pro Tyr Tyr Trp His Trp Ile Arg Thr Tyr Ile His His Gly
65 70 75 80

Val Arg Thr Gly Lys Ile Asp Leu Glu Glu Leu Glu Arg Arg Thr Gln
85 90 95

Tyr Tyr Arg Glu Asn Pro Asp Ala Pro Leu Pro Glu His Glu Gln Lys
100 105 110

Pro Glu Leu Ile Glu Phe Val Asn Gln Ala Val Tyr Gly Gly Leu Pro 115 120 125

Ala Ser Arg Glu Val Asp Arg Pro Pro Lys Phe Lys Glu Gly Asp Val 130 135 140

Val Arg Phe Ser Thr Ala Ser Pro Lys Gly His Ala Arg Arg Ala Arg 145 150 155 160

Tyr Val Arg Gly Lys Thr Gly Thr Val Val Lys His His Gly Ala Tyr 165 170 175

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cgcgcgtggt acgtgcgc
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      primer
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Ile Thr Ala Arg Val Lys Ala Leu Glu Ser Met Leu Ile Glu Gln Gly
Ile Leu Thr Thr Ser Met Ile Asp Arg Met Ala Glu Ile Tyr Glu Asn
Glu Val Gly Pro His Leu Gly Ala Lys Val Val Lys Ala Trp Thr
Asp Pro Glu Phe Lys Lys Arg Leu Leu Ala Asp Gly Thr Glu Ala Cys
 65
Lys Glu Leu Gly Ile Gly Gly Leu Gln Gly Glu Asp Met Met Trp Val
Glu Asn Thr Asp Glu Val His His Val Val Val Cys Thr Leu Cys Ser
            100
                                105
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Cys Tyr Pro Trp Pro Val Leu Gly Leu Pro Pro Asn Trp Phe Lys Glu 115 120 125

Pro Gln Tyr Arg Ser Arg Val Val Arg Glu Pro Arg Gln Leu Leu Lys 130 135 140

Glu Glu Phe Gly Phe Glu Val Pro Pro Ser Lys Glu Ile Lys Val Trp 145 150 155 160

Asp Ser Ser Ser Glu Met Arg Phe Val Val Leu Pro Gln Arg Pro Ala 165 170 175

Gly Thr Asp Gly Trp Ser Glu Glu Glu Leu Ala Thr Leu Val Thr Arg 180 185 190

Glu Ser Met Ile Gly Val Glu Pro Ala Lys Ala Val Ala 195 200 205

<210> 99

<211> 233

<212> PRT

<213> Pseudonocardia thermophila

<400> 99

Met Asn Gly Val Tyr Asp Val Gly Gly Thr Asp Gly Leu Gly Pro Ile

1 10 15

Asn Arg Pro Ala Asp Glu Pro Val Phe Arg Ala Glu Trp Glu Lys Val 20 25 30

Ala Phe Ala Met Phe Pro Ala Thr Phe Arg Ala Gly Phe Met Gly Leu
35 40 45

Asp Glu Phe Arg Phe Gly Ile Glu Gln Met Asn Pro Ala Glu Tyr Leu 50 55 60

Glu Ser Pro Tyr Tyr Trp His Trp Ile Arg Thr Tyr Ile His His Gly
65 70 75 80

Val Arg Thr Gly Lys Ile Asp Leu Glu Glu Leu Glu Arg Arg Thr Gln 85 90 95

Tyr Tyr Arg Glu Asn Pro Asp Ala Pro Leu Pro Glu His Glu Gln Lys
100 105 110

Pro Glu Leu Ile Glu Phe Val Asn Gln Ala Val Tyr Gly Gly Leu Pro 115 120 125

Ala Ser Arg Glu Val Asp Arg Pro Pro Lys Phe Lys Glu Gly Asp Val 130 135 140

Val Arg Phe Ser Thr Ala Ser Pro Lys Gly His Ala Arg Arg Ala Arg 145 150 155 160

Tyr Val Arg Gly Lys Thr Gly Thr Val Val Lys His His Gly Ala Tyr 165 170 175

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Ile Tyr Pro Asp Thr Ala Gly Asn Gly Leu Gly Glu Cys Pro Glu His
           180
                               185
Leu Tyr Thr Val Arg Phe Thr Ala Gln Glu Leu Trp Gly Pro Glu Gly
Asp Pro Asn Ser Ser Val Tyr Tyr Asp Cys Trp Glu Pro Tyr Ile Glu
   210
                       215
Leu Val Asp Thr Lys Ala Ala Ala Ala
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gtcaaggccc tggagtcgat gctcatcgaa cagggcatcc tcaccacgtc gatgatcgac 120
cggatggccg agatctacga gaacgaggtc ggcccgcacc tcggcgcgaa ggtcgtcgtg 180 ^{\circ}
aaggootgga cogacoogga gttcaagaag ogtotgotog cogacoggoac ogaggootgo 240
aaggageteg geateggegg eetgeaggge gaggacatga tgtgggtgga gaacacegae 300
gaggtccacc acgtcgtcgt gtgcacgctc tgctcctgct acccgtggcc ggtgctgggg 360
ctgccgccga actggttcaa ggagccgcag taccgctccc gcgtggtgcg tgagccccgg 420
cagctgctca aggaggagtt cggcttcgag gtcccgccga gcaaggagat caaggtctgg 480
gactccagct ccgagatgcg cttcgtcgtc ctcccgcagc gccccgcggg caccgacggg 540
tggagcgagg aggagctcgc caccctcgtc acccgcgagt cgatgatcgg cgtcgaaccg 600
gcgaaggcgg tcgcgtga
                                                                618
<210> 101
<211> 702
<212> DNA
<213> Pseudonocardia thermophila
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gacgaaccgg tetteegege egagtgggag aaggtegegt tegegatgtt eeeggegaeg 120
ttccgggccg gcttcatggg cctggacgag ttccggttcg gcatcgagca gatgaacccg 180
gccgagtacc tcgagtcgcc gtactactgg cactggatcc gcacctacat ccaccacggc 240
gtecgcaccg gcaagatcga tetegaggag etggagegee gcaegcagta etacegggag 300
aaccccgacg ccccgctgcc cgagcacgag cagaagccgg agttgatcga gttcgtcaac 360
caggeegtet aeggegget geeegeaage egggaggteg aeegaeegee caagtteaag 420
tacgtgcgcg gcaagaccgg gacggtggtc aagcaccacg gcgcgtacat ctacccggac 540
accgccggca acggcctggg cgagtgcccc gagcacctct acaccgtccg cttcacggcc 600
caggagctgt gggggccgga aggggacccg aactccagcg tctactacga ctgctgggag 660
ccctacatcg agctcgtcga cacgaaggcg gccgcggcat ga
                                                                 702
<210> 102
<211> 144
<212> PRT
<213> Pseudonocardia thermophila
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Arg Ala Leu Asp Arg Gly Phe Asp Glu Pro Trp Gln Leu Arg Ala Phe 35 40 45

Ala Leu Ala Val Ala Ala Cys Arg Ala Gly Arg Phe Glu Trp Lys Gln 50 55 60

Leu Gln Gln Ala Leu Ile Ser Ser Ile Gly Glu Trp Glu Arg Thr His 65 70 75 80

Asp Leu Asp Asp Pro Ser Trp Ser Tyr Tyr Glu His Phe Val Ala Ala 85 90 95

Leu Glu Ser Val Leu Gly Glu Glu Gly Ile Val Glu Pro Glu Ala Leu 100 105 110

Asp Glu Arg Thr Ala Glu Val Leu Ala Asn Pro Pro Asn Lys Asp His 115 120 125

His Gly Pro His Leu Glu Pro Val Ala Val His Pro Ala Val Arg Ser 130 135 140

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<210> 103
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<212> DNA
<213> Pseudonocardia thermophila
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<400> 103

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rtgagcgccgaggcgaaggtccgcctgaagcactgcccacggccgaggaccggcggcggccgacgcctgctcgcgagctgcccggggcgaccgcgcgctcgaccgcgcttcgaccggagccgtggagctgcggcgttcgcgctggggtcgcggcgtgcagggggcccggttcgagtggaagcagctgcagcaggcgctgatctcctcgatcggggagtggagcgcacccacgatctcgacgaccgagctggtcctactacgagcacttcgtcgccgcgtggaatccggctcggcgaggaagggatcgtcgagccggaggcgctggacgagcgcacccacgaggtcttggccaacccgcgaacaaggatcaccatggaccgcatctgagcccgtcgggtccaccc420

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<210> 104
<211> 1315
<212> DNA
<213> Rhodococcus rhodochrous
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<221> CDS
<222> (1)..(690)
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<220>

<221> CDS <222> (704)..(1315) <400> 104 48 atg gat ggt atc cac gac aca ggc ggc atg acc gga tac gga ccg gtc Met Asp Gly Ile His Asp Thr Gly Gly Met Thr Gly Tyr Gly Pro Val ccc tat cag aag gac gag ccc ttc ttc cac tac gag tgg gag ggt cgg 96 Pro Tyr Gln Lys Asp Glu Pro Phe Phe His Tyr Glu Trp Glu Gly Arg acc ctg tca att ctg act tgg atg cat ctc aag ggc ata tcg tgg tgg 144 Thr Leu Ser Ile Leu Thr Trp Met His Leu Lys Gly Ile Ser Trp Trp 35 40 gac aag tcg cgg ttc ttc cgg gag tcg atg ggg aac gaa aac tac gtc 192 Asp Lys Ser Arg Phe Phe Arg Glu Ser Met Gly Asn Glu Asn Tyr Val aac gag att cgc aac tcg tac tac acc cac tgg ctg agt gcg gca gaa 240 Asn Glu Ile Arg Asn Ser Tyr Tyr Thr His Trp Leu Ser Ala Ala Glu cgt atc ctc gtc gcc gac aag atc atc acc gaa gaa gag cga aag cac 288 Arg Ile Leu Val Ala Asp Lys Ile Ile Thr Glu Glu Glu Arg Lys His cgt gtg caa gag atc ctt gag ggt cgg tac acg gac agg aag ccg tcg 336 Arg Val Gln Glu Ile Leu Glu Gly Arg Tyr Thr Asp Arg Lys Pro Ser 105 cgg aag ttc gat ccg gcc cag atc gag aag gcg atc gaa cgg ctt cac 384 Arg Lys Phe Asp Pro Ala Gln Ile Glu Lys Ala Ile Glu Arg Leu His 120 125 gag ccc cac tcc cta gcg ctt cca gga gcg gag ccg agt ttc tct ctc 432 Glu Pro His Ser Leu Ala Leu Pro Gly Ala Glu Pro Ser Phe Ser Leu ggt gac aag atc aaa gtg aag agt atg aac ccg ctg gga cac aca cgg 480 Gly Asp Lys Ile Lys Val Lys Ser Met Asn Pro Leu Gly His Thr Arg 145 tgc ccg aaa tat gtg cgg aac aag atc ggg gaa atc gtc gcc tac cac Cys Pro Lys Tyr Val Arg Asn Lys Ile Gly Glu Ile Val Ala Tyr His 165 175 gge tge cag ate tat eee gag age age tee gee gge ete gge gae gat Gly Cys Gln Ile Tyr Pro Glu Ser Ser Ala Gly Leu Gly Asp Asp 180 185 cct cgc ccg ctc tac acg gtc gcg ttt tcc gcc cag gaa ctg tgg ggc Pro Arg Pro Leu Tyr Thr Val Ala Phe Ser Ala Gln Glu Leu Trp Gly 195 200

					aaa Lys										672
	ctg Leu				tgaa	aagga	aat a	acgat						at aag sn Lys	724
	_				gca Ala	_				_		_	_		772
					acg Thr										820
					ggc Gly 45										868
					gag Glu										916
_		_			ttg Leu			_	 	_	_				964
_		_			gac Asp			_					_		1012
					ccg Pro										1060
					tac Tyr 125										1108
		_	_	_	ttc Phe			_		_				_	1156
					agc Ser										1204
					gac Asp										1252
					atg Met										1300

gaa gtg atc gta tga Glu Val Ile Val 200	131
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gttttcccag tcacgac	17
<210> 108	
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<222> (14)..(16)
<223> a, c, g, t, unknown or other
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<223> a, c, g, t, unknown or other
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                                                                 . 18
<210> 112
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<223> Description of Artificial Sequence: Synthetic
      primer
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<222> (7)..(9)
<223> a, c, g, t, unknown or other
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<223> a, c, g, t, unknown or other
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aagaagnnnc tgctcgcc
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<223> a, c, g, t, unknown or other
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<220>
<221> modified_base
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<223> a, c, g, t, unknown or other
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ctcgccnnnc tcgtcact
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<223> a, c, g, t, unknown or other
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     primer
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<223> a, c, g, t, unknown or other
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<210> 125
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<223> a, c, g, t, unknown or other
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      primer
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     primer
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<223> a, c, g, t, unknown or other
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ggcgcgnnna tctacccg
                                                                   18
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aacggcnnng gcgagtgc
                                                                   18
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tactacnnnt gctgggag
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tacgacnnnt gggagccc
                                                                   18
<210> 140
<211> 229
<212> PRT
<213> Rhodococcus rhodochrous
<400> 140
Met Asp Gly Ile His Asp Thr Gly Gly Met Thr Gly Tyr Gly Pro Val
Pro Tyr Gln Lys Asp Glu Pro Phe Phe His Tyr Glu Trp Glu Gly Arg
             20
                                 25
Thr Leu Ser Ile Leu Thr Trp Met His Leu Lys Gly Ile Ser Trp Trp
Asp Lys Ser Arg Phe Phe Arg Glu Ser Met Gly Asn Glu Asn Tyr Val
     50
Asn Glu Ile Arg Asn Ser Tyr Tyr Thr His Trp Leu Ser Ala Ala Glu
                     70
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Arg Ile Leu Val Ala Asp Lys Ile Ile Thr Glu Glu Glu Arg Lys His
85 90 95

Arg Val Gln Glu Ile Leu Glu Gly Arg Tyr Thr Asp Arg Lys Pro Ser

Arg Lys Phe Asp Pro Ala Gln Ile Glu Lys Ala Ile Glu Arg Leu His
115 120 125

Glu Pro His Ser Leu Ala Leu Pro Gly Ala Glu Pro Ser Phe Ser Leu 130 135 140

Gly Asp Lys Ile Lys Val Lys Ser Met Asn Pro Leu Gly His Thr Arg 145 150 155 160

Cys Pro Lys Tyr Val Arg Asn Lys Ile Gly Glu Ile Val Ala Tyr His
165 170 175

Gly Cys Gln Ile Tyr Pro Glu Ser Ser Ser Ala Gly Leu Gly Asp Asp 180 185 190

Pro Arg Pro Leu Tyr Thr Val Ala Phe Ser Ala Gln Glu Leu Trp Gly 195 200 205

Asp Asp Gly Asn Gly Lys Asp Val Val Cys Val Asp Leu Trp Glu Pro 210 215 220

Tyr Leu Ile Ser Ala 225

<210> 141

<211> 203

<212> PRT

<213> Rhodococcus rhodochrous

<400> 141

Met Ser Glu His Val Asn Lys Tyr Thr Glu Tyr Glu Ala Arg Thr Lys

1 10 15

Ala Ile Glu Thr Leu Leu Tyr Glu Arg Gly Leu Ile Thr Pro Ala Ala 20 25 30

Val Asp Arg Val Val Ser Tyr Tyr Glu Asn Glu Ile Gly Pro Met Gly 35 40 45

Gly Ala Lys Val Val Ala Lys Ser Trp Val Asp Pro Glu Tyr Arg Lys 50 55 60

Trp Leu Glu Glu Asp Ala Thr Ala Ala Met Ala Ser Leu Gly Tyr Ala 65 70 75 80

Gly Glu Gln Ala His Gln Ile Ser Ala Val Phe Asn Asp Ser Gln Thr 85 90 95

His His Val Val Cys Thr Leu Cys Ser Cys Tyr Pro Trp Pro Val

Leu Gly Leu Pro Pro Ala Trp Tyr Lys Ser Met Glu Tyr Arg Ser Arg
115 120 125

Val Val Ala Asp Pro Arg Gly Val Leu Lys Arg Asp Phe Gly Phe Asp 130 135 140

Ile Pro Asp Glu Val Glu Val Arg Val Trp Asp Ser Ser Glu Ile
145 150 155 160

Arg Tyr Ile Val Ile Pro Glu Arg Pro Ala Gly Thr Asp Gly Trp Ser 165 170 175

Glu Glu Glu Leu Thr Lys Leu Val Ser Arg Asp Ser Met Ile Gly Val 180 185 190

Ser Asn Ala Leu Thr Pro Gln Glu Val Ile Val 195 200

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-22N N

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